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Original Article

# PREVALENCE OF SUB - CLINICAL MASTITIS IN AREAS AROUND LAKHIMPUR TOWN OF ASSAM

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***ABSTRACT***

***Mastitis poses a serious threat to the economics of the dairy industry since it affects both the quality and the quantity of milk produced. The subclinical form can be more challenging since it remains largely undiagnosed, persists for longer duration and is more prevalent than the clinical form. The present study, therefore, was undertaken to assess the prevalence of subclinical mastitis in local and cross bred cows of unorganized farms located in and around North Lakhimpur town of the state of Assam. Milk samples from apparently mastitis free 368 quarters of 92 cows belonging to different herds were collected for this study following standard protocol. The samples were subjected to California Mastitis Test (CMT), Modified Whiteside Test (MWT) and pH test. The pH meter readings of the milk samples could not yield any conclusive difference between the samples. The samples exhibiting gel formation in CMT and MWT were considered as positive for subclinical mastitis. A total number of 18 (19.56 %) cows were found to be CMT positive with all the four quarters showing positive reaction. On quarter basis, 72 (19.56 %) quarters were found to be affected. Similar results were obtained using both the tests. Primary isolation from the positive samples were done on Blood agar followed by subculture on Mac Conkey agar, Eosin Methylene Blue Agar and Baird Parker Agar plates, supporting the growth of various types of bacteria for their study and isolation. Bacterial identification was based on their morphology and motility, growth characteristics on the culture medium and biochemical characteristics. Out of the 18 positive samples Klebsiella pneumoniae could be isolated from 11 samples (61.11 %), followed by Escherichia coli from 7 samples (38.88%), coagulase negative Staphylococcus spp. from 5 samples (27.77%) and Proteus spp. from 3 (16.66%).***

***KEYWORDS:*** ***Subclinical Mastitis, California Mastitis Test, Modified White Side Test, Klebsiella, Escherichia Coli, & Staphylococcus***

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## INTRODUCTION

Mastitis is a serious problem in dairy herds resulting in enormous losses in terms of production. The losses are due to reduced milk yield, inferior milk quality, premature culling, additional labour and expenses on treatment or control measures. (Radostits *et al*. 2000; Hogeveen *et al*. 2011). Mastitis can be defined as the inflammation of the mammary gland and is primarily classified into two forms -clinical and subclinical mastitis (Kader *et al.* 2003). Clinical mastitis can be easily diagnosed on the basis of visible abnormalities

(Sinha *et. al*., 2014; Kurjogi *et. al*., 2014). However, no gross changes are observed in subclinical mastitis, making laboratory diagnosis (Kurjogi *et. al*., 2014) along with regular testing (Hegde *et. al*., 2013) imperative for its detection.

Milk production is affected by both forms of mastitis and it has been estimated that the average decrease in milk yield due to clinical and subclinical mastitis was 50 and 17.5 %, respectively (Singh and Singh, 1994) Reports, however, suggest that subclinical mastitis has a higher prevalence than clinical mastitis (Seegers *et al*. 2003; Mdegela *et al*. 2009). In one study conducted in India, subclinical mastitis was found to be more important (10–50% in cows and 5–20% inbuffaloes) than clinical mastitis (1–10%) (Joshi and Gokhale, 2006). Studies indicate that the economic loss due to both forms of mastitis has increased almost five-fold from Rs. 16,072 millions (Singh and Singh, 1994) to Rs. 71,655 millions (Bansal and Gupta, 2009) in a span of just 15 years.

Another alarming aspect of mastitis is the increased risk of transmission of communicable diseases such as tuberculosis, brucellosis, staphylococcal toxemia, septic sore throat, gastroenteritis etc. (Radostits *et al*., 2000; Jeykumar *et. al*. 2013). A wide range of microorganisms have been implicated in the production of mastitis (Kayesh *et. al*., 2014; Jeykumar *et. al*. 2013). Among these mastitogens *Staphylococcus aureus*, *Streptococcus agalactiae*, *Strep. dysgalactiae*, *Strep. uberis* and *Escherichia coli* have been considered to be important (Hegde *et. al*., 2013 ).

Earlier research works on various aspects of bovine mastitis have shown variability in the prevalence pattern of mastitis. Therefore, further extensive screening of cattle herds is of paramount importance for formulating therapeutic and control measures for bovine mastitis in a country like India with diverse agroclimatic conditions. Early diagnosis, particularly of subclinical mastitis, is vital since it remains unnoticed but brings about unwanted changes in the milk. For this study, unorganized farms located in and around Lakhimpur town of Assam were selected since previous reports relating to the prevalence of SCM are lacking or scanty from this part of the country.

## MATERIALS AND METHODS:

### Sample Collection

The study was conducted on a total of 92 apparently healthy cows of which 37 were local breed while 55 were cross bred. Milk samples, of approximately 10 ml, were collected following strict aseptic measures from the four quarters of the cows (n=368). Briefly, the udder was wiped clean with potassium permanganate solution (1:1000), allowed to dry and mid-stream milk was sampled (Jeykumar *et. al*. 2013).

### Screening of Samples for Detection of Subclinical Mastitis

The quarter milk samples (n=368) were subjected to California Mastitis Test (CMT) following standard procedure (Abdel-Rady and Sayed, 2009). The visible reaction observed in CMT was graded from 0 to 3 depending upon the degree of gelation: 0 = negative or traces (no change in consistency), 1 = slightly positive (+), 2 = positive (++) and 3 = highly positive (+++). Similarly, all the samples were also screened by Modified Whiteside Test following the procedure described by Easterday *et. al*., 1958.

### Determination of the PH

In order to check the alkalinity of milk samples, pH readings were recorded using a pH meter following the manufacturer's instructions.

**Bacterial Isolation**

The pooled milk samples of the CMT positive cows were processed for bacterial isolation. The pooled samples were immediately transported to the laboratory maintaining cold chain for further investigation. The milk samples were initially inoculated into Blood Agar containing 5 percent defibrinated sheep blood and Mac Conkey's Lactose Agar (Himedia Laboratories Ltd). The plates were then incubated at 37°C for 24-48 hours. Further, suspected colonies were subcultured onto Eosin Methylene Blue Agar and Baird-Parker agar. Preliminary identification of the bacteria was done on the basis of their morphology, staining reaction, motility and cultural characteristics. For confirmation, the bacterial isolates were subjected to a series of biochemical tests *viz.* IMViC, Catalase, Coagulase, sugar fermentation tests etc.

## RESULTS AND DISCUSSIONS

In the present investigation, it was observed that milk samples of 18 (19.56 %) cows were positive in the California mastitis test. In our study Modified White side Test was also employed alongside CMT for detection of subclinical mastitis and it was found to equally effective. California mastitis test (CMT) and Modified White Side test (MWT) are among the various screening tests preferred for detection of subclinical mastitis as they can be used easily, yielding rapid as well as satisfied results (Lesile *et al*., 2002). However, several investigations have used CMT because it has been found to be more perfect, efficient and reliable than other field and chemical tests for diagnosis of subclinical mastitis (Viani *et al*., 1990; Behera and Dwivedi, 1992; El- Balkemy *et. al*., 1997). While comparing the two tests, Joshi and Gokhale (2006) observed that MCMT was more efficacious (96.73%) than MWST (89.08%) in diagnosis of subclinical mastutis. In another study, CMT and MWT scores were found to be in agreement in 56.4% of samples including 87.5% of samples with high (+++) scores (Cho and Cheong, 1970)

The pH meter readings showed that the pH of the quarter milk samples collected during the study were within the acceptable limits irrespective of them being positive or negative in CMT. Contrary to our findings, different workers have reported significantly higher pH in subclinical mastitic milk than in the normal ones. (Batavani *et. al*., 2007; Sena and Sahmani, 2001; Wielgosz-Groth and Groth, 2003). The pH of normal cow's milk ranges between 6.5 to 6.8 and studies suggest that it may exceed to upto 7.0 in milks with high cell counts (Marschke and Kitchen, 1985). Somatic cell count (SCC) has been regarded as a useful predictor of subclinical mastitis (Harmon, 1994) since it is characterized by an increase in somatic cells in the milk (Kayesh et. al., 2014). In this context, Atasever *et. al* (2010) tried to assess the relationship between SCC and pH in raw bovine milk and concluded that pH records of raw milk are not suggested to determine subclinical mastitis or quality of milk. Similarly, in another study, it was found that SCC had no significant effect on the pH values of quarter milk samples, with the mean pH values falling within the normal milk pH range (Ogola *et. al*, 2007)

Among the 18 (19.56 %) cows found to be affected with subclinical mastitis, it was observed that 16 (29.09 %) out of the 55 cross bred cows were positive while only 2 (5.4 %) out of the 37 cows of local breed were positive. Bangar *et. al*. (2015) observed that the prevalence of subclinical mastitis on cow basis varied from 20.73 to 78.55 %. Also, high incidences of subclinical mastitis have been reported from various parts of the country with figures ranging from 18.40%7 to 72.60% in cross bred cows and 15.78% to 81.60%, in local cows (Joshi and Gokhale, 2006). In this study only 5.4 % of the local cows were found to be affected by sub clinical mastitis which is comparable with earlier reports suggesting that the incidence among local cattle and buffaloes was the lowest while it was highest among pure-bred Holsteins and Jerseys (NAAS, 2013).

On quarter-basis, 72 (19.56 %) were found to be affected since all the four quarters of the 18 cows were CMT positive. Notably, the degree of gel formation in the CMT positive quarter milk samples was found to be variable. The details of the CMT score of the positive quarter milk samples are presented in Table no 1. Kayesh *et. al*., (2014) too had earlier reported a variation of CMT score even in different quarters of individual lactating cattle.

Infection of all the four quarters observed in our study is in contrary to previous studies which have described dissimilarity in the infection rate. Joshi and Gokhale (2006) found that infection with one, two, three, and four quarters was 45.21%, 35.65%, 13.04% and 6.08% respectively. They also concluded that the incidence was found more in hind quarters (56.52%) than fore quarters (43.47%). Similarly, Belina *et al* (2016) too reported higher infection rate in hindquarters as compared to the front quarters. Shittu *et al* (2012) on the other hand observed that the Left Fore Quarters were more affected than the other quarters. The variation in the incidence has been attributed to various factors viz. management practices, cattle population, stage and number of lactations, environment, breed, nutritional status, size of herd, year of study, region, and zoogeography (Radostits *et. al*., 2000 ). In this case infection of all the quarters might have resulted due to unhygienic or improper milking. It has been suggested that one quarter is usually first infected and the others become affected through contamination and other means especially during the milking procedures (Shittu *et al.*,2012)

Bacteriological examination of the milk samples collected from the 18 CMT positive cows revealed presence of *Klebsiella pneumonia, Escherichia coli*, coagulase negative *Staphylococcus spp.* and *Proteus spp.* The details are presented in Table no. 2. All the 18 milk samples were found to be positive for atleast one bacteria. Two or three different types of bacteria could be isolated from 6 milk samples. In the present study, *Klebsiella pneumoniae* was found to be the most prominent bacterial agent as it could be isolated from 11 samples (61.11 %). Furthermore, *Escherichia coli* was isolated from 7 samples (38.88%) followed by coagulase negative *Staphylococcus spp.* from 5 samples (27.77%) and *Proteus spp.* from 3 (16.66%). Earlier reports describing isolation of *Klebsiella spp* from mastitic milk indicate presence in less than 10 percent of the samples (Sharma and Sindhu, 2007; Ranjan et. al. 2011; Jeykumar *et. al*. 2013). Instead a higher prevalence of *Staphylococcus* spp. followed by *Streptococcus* spp. and *Escherichia coli* has been reported by different workers in both clinical and subclinical mastitis (Kayesh *et al*., 2014, Sanotharan *et. al*., 2016).

In this study, presence of only coagulase negative *Staphylococcus spp* was evident in contrast to the findings of Hegde *et. al*., 2013 who could isolate both *S. aureus* and coagulase negative staphylococci from the milk of subclinically infected cattle. The findings of our study pertaining to *Proteus spp* isolation are comparable to that of Belina *et. al*., 2016 who reported isolation of *Proteus spp* from 1.9 % of cow milk samples found positive in CMT. In another study involving buffaloes affected with clinical and subclinical mastitis *Proteus spp* was identified from 0.14% of the samples (Sharma and Sindhu, 2007)

Our results have shown a predominance of *Klebsiella spp.* and *E. coli*. In this context Iraguha *et. al*., 2015, had opined that high percentage of mastitis caused by coliform bacteria indicated contamination from soil and faecal matter. In another report, faecal shedding of *K. pneumoniae* by a large proportion of dairy cows was considered as the reason for occurrence of *Klebsiella* mastitis in herds (Munoz *et. al*, 2006). Notably, higher incidence of *Staphylococcus aureus*, *Streptococcus uberis*,coagulase-negative staphylococci and other streptococcal infections has been reported from tie-stalls in comparison to free-stalls where *Klebsiella* sp. and *E. coli* were the main concerns (NAAS, 2013). All the samples for this study were collected from un-organised farms where proper animal husbandry procedures were not employed and a lack of hygiene may explain the higher occurrence of environmental pathogens.

## CONCLUSIONS

The results of the current investigation provide useful data on the status of subclinical mastitis in the vicinity of North Lakhimpur town of Assam, which has remained largely unexplored in the area of dairy production. In this study, 19.56 % of the cows were found to be affected with subclinical mastitis. The prevalence was higher among cross bred cows 16 (29.09 %) in comparison to local cows 2 (5.4 %). Isolation attempts revealed occurrence of different pathogens in the milk of the affected animals which underlines the need to educate the farmers on the importance of sanitation to prevent the occurrence of the disease. Isolation of mastitis pathogens also highlights the related aspects of public health and food safety. Rural farmers in this part of the country practice traditional methods of livestock farming. An effective system has to be developed to create awareness among the farmers for adoption of scientific management procedures and preventive measures to limit mastitis related losses, prevent further transmission and enhance quality of milk produced.

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## APPENDICES

**Table 1: CMT Scores of the Different Quarters of the Individual Cows**

| Sl. No. | RH | LH | RF | LF |
|---|---|---|---|---|
| 1 | 2+ | 1+ | 1+ | 1+ |
| 2 | 3+ | 1+ | 2+ | 1+ |
| 3 | 2+ | 1+ | 1+ | 1+ |
| 4 | 1+ | 2+ | 1+ | 1+ |
| 5 | 2+ | 1+ | 1+ | 1+ |
| 6 | 2+ | 3+ | 2+ | 1+ |
| 7 | 1+ | 1+ | 2+ | 1+ |
| 8 | 2+ | 2+ | 1+ | 1+ |
| 9 | 2+ | 3+ | 2+ | 1+ |
| 10 | 2+ | 2+ | 1+ | 2+ |
| 11 | 1+ | 1+ | 3+ | 1+ |
| 12 | 2+ | 2+ | 3+ | 1+ |
| 13 | 2+ | 3+ | 1+ | 2+ |
| 14 | 3+ | 3+ | 2+ | 2+ |
| 15 | 2+ | 2+ | 2+ | 2+ |
| 16 | 2+ | 2+ | 2+ | 2+ |
| 17 | 2+ | 2+ | 2+ | 1+ |
| 18 | 2+ | 1+ | 2+ | 1+ |

*RH- Right hind, LH- Left hind, RF- Right fore, LF- Left fore

**Table 2: Isolation of Different Bacteria from CMT Positive Milk Samples**

| Samples for Bacteriological Culture (Numbers) | Bacterial Isolates | Number of Isolates | Prevalence (%) |
|---|---|---|---|
| 18 | Klebsiella pneumoniae | 11 | 61.11 |
| | Escherichia coli | 7 | 38.88 |
| | Staphylococcus spp | 5 | 27.77 |
| | Proteus spp | 3 | 16.66 |